Onchocerca–Simulium complexes in Venezuela: can human onchocerciasis spread outside its present endemic areas?

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SUMMARY

The compatibility between sympatric and allopatric combinations of *Onchocerca volvulus*–anthropophilic species of *Simulium* was studied in the north-eastern focus of human onchocerciasis as well as in a densely populated locality of the Amazonas State in Venezuela. The objectives were to test the conjecture that local adaptation exists between the parasite and its vectors (the *Onchocerca–Simulium* complex hypothesis), and assess the possibility of the infection spreading from its present distributional range. For the homologous combination, *O. volvulus–S. metallicum* cytospecies E in Anzoátegui State (north-eastern focus), parasite yield was 45 % in contrast to 1 % for the heterologous, southern parasite–*S. metallicum* infection. This was significantly lower than the parasite yield (4–10 %) expected after allowing for the effect of density-dependent limitation of infective larval output described in this paper for *S. metallicum*. The population of *S. exiguum s.l.* from southern Venezuela allowed no larval development beyond the L1 stage of either northern or southern parasites. Mechanisms for such refractoriness probably operate at the level of the thoracic muscles, not affecting microfilarial uptake or migration out of the bloodmeal. The parasite yield of southern *O. volvulus* in *S. oyapockense s.l.* flies biting man at Puerto Ayacucho (Amazonas) was about 1 %, in agreement with the figures recorded for highly compatible sympatric combinations such as *O. volvulus–S. ochraceum s.l.* in Guatemala. No infective larval development of the northern parasite was observed in southern *S. oyapockense*. These results, together with considerations of typical worm burdens in the human host, presence/absence of armed cibaria in the simuliids, parasite-induced vector mortality, and fly biting rates, suggest a lower potential for onchocerciasis to spread between the northern and southern endemic areas of Venezuela than that between Amazonian hyperendemic locations and settlements outside this focus with high densities of *S. oyapockense s.l.*

Key words: onchocerciasis spread, cross-infection experiments, local adaptation, *Simulium metallicum s.l.*, *S. exiguum s.l.*, *S. oyapockense s.l.*, Venezuela.

INTRODUCTION

Two main areas endemic for human onchocerciasis exist in Venezuela, the former situated in the north-central (Arends, Rondón & González, 1954) and north-eastern (Potenza, Febres-Cordero & Anduze, 1948) mountain ranges of the country (the Altamira and Caripe foci, respectively), and the latter in the southern Amazonian region (the Sierra Parima highlands and Upper Orinoco lowlands), close to the border with Brazil (Rassi *et al.* 1977; Basáñez & Yarzábal, 1989). In the northern area the infection is transmitted mainly by *Simulium metallicum* Bellardi *s.l.* (Peñalver, 1961; Lewis & Ibáñez de Aldecoa, 1962; Grillet *et al.* 1994), whereas in the Amazonian focus, *S. guianense* Wise *s.l.* (often referred to as *S. pintoi*), *S. oyapockense* Floch and Abonnenc *s.l.*/*S. roraimense* Nunez de Mello (also referred to as *S. amazonicum* and *S. cuasisanguineum*), and possibly *S. incrustatum* Lutz/*S. limbatum* Knab (as *S. yarzabali*) are the vectors (Rassi *et al.* 1977; Takaoka *et al.* 1984*a*; Basáñez *et al.* 1988; Shelley *et al.* 1997). *S. exiguum* Roubaud *s.l.* occurs in both foci but, whereas it might play a minor role in the north (Duke, 1970), its man-biting rate within the southern focus seems too low to warrant vectorial status (Vivas-Martínez *et al.* 1998). However, outside the southern onchocerciasis area, the man-seeking density of *S. exiguum s.l.* may reach very high levels. Members of this species complex play a major vectorial role in Ecuador (Shelley & Arzube, 1985; Shelley, Procunier & Arzube, 1986), being the only species transmitting *Onchocerca volvulus* Leuckart in Colombia (Tidwell *et al.* 1980; Corredor *et al.* 1998). *S. oyapockense/roraimense* bites man in high numbers along the main river systems of the Amazonas region of Venezuela, acting as a vector of *Mansonella ozzardi* Manson in the middle reaches of the Orinoco river

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(as *S. sanchezi* (Yarzábal *et al*. 1985)) and of *O. volvulus* in the Upper Orinoco region (M. E. Grillet, unpublished observations).

The notion of the existence of well-adapted *Onchocerca–Simulium* complexes, initially proposed by Duke, Lewis & Moore (1966) in Cameroon, and later confirmed elsewhere in Africa (Philippon, 1977), was based on the observation that microfilariae (mf) of West African forest parasites developed efficiently in *S. damnosum* from West African forest but poorly or not at all in Sudan-savanna flies. Conversely, the success of Sudan-savanna parasites was high when developing within savanna *S. damnosum* but significantly reduced within forest flies. Later, reciprocal cross-infection studies between West Africa and Guatemala (De León & Duke, 1966; Duke, Moore & De León, 1967), and West Africa and northern Venezuela (Duke, 1970), documented that homologous parasite–vector combinations (i.e. from the same geographical region) were more successful than heterologous ones (from allopatric endemic areas).

In an evolutionary context, host–parasite interactions are expected to result in geographical patterns of adaptation in which parasites are better able to infect their local host populations (Ebert, 1994; Lively & Jokela, 1996). This has been confirmed by recent experimental infection studies across a wide variety of host–parasite systems (Parker, 1985; Lively, 1989; Failloux *et al*. 1995). However, non-significant differences have been found between Guatemalan and North Venezuelan *O. volvulus* populations in their ability to infect local blackfly vectors (*S. ochraceum*, *S. metallicum*, *S. callidum* and *S. haematopotum* in Guatemala, and *S. metallicum* in Venezuela (Takaoka *et al*. 1986*a*)), confirming earlier observations (Duke, 1970). This was interpreted as close genetic proximity between these two parasite populations (Takaoka *et al*. 1986*b*).

More recent work, based on analysis of a tandemly repeated DNA sequence family present in the genome of *O. volvulus* (O–150) has confirmed that West African rainforest and savanna parasite populations are indeed significantly different and that some barrier preventing genetic exchange between these two populations must have developed, the existence of vector–parasite transmission complexes being one of several possible explanations (Zimmerman *et al*. 1994). However, profound changes in the epidemiological and ecological landscape of West Africa in the last 20 years, partially due to the Onchocerciasis Control Program (OCP), have apparently disrupted such complexes, with important consequences regarding possible recrudescence following cessation of vector control (Toé *et al*. 1997).

If highly compatible *Onchocerca–Simulium* complexes exist within each undisturbed main endemic area, the question arises as to the potential for onchocerciasis to spread towards new regions where infected carriers settle and high densities of anthropophilic simuliids occur. This question seems particularly relevant in Neotropical settings, where, coupled with wide distribution of potential vectors, there is increasing population mobility to and from established foci due to mounting economic and social pressures to develop inland areas (Anon, 1986). Evidence is now emerging from Brazil and Ecuador of locally acquired onchocercal infections, or potential for this to take place, outside original endemic areas (Gerais & Ribeiro, 1986; Guderian & Shelley, 1992; Charalambous, Shelley & Arzube, 1997). Laurence & Pester (1967) described the relatively rapid adaptation of the filarial worm, *Brugia patei*, to a new mosquito host, *Aedes togoi*, in the laboratory and discussed the implications of this finding in explaining present distribution of filariases.

In this paper we explore the ability of *O. volvulus* to develop in sympatric and allopatric man-biting blackfly species of northern and southern Venezuela in order to, first, test the conjecture that *Onchocerca–Simulium* complexes may exist within Venezuelan endemic areas, and second, investigate the vector competence of highly anthropophilic simuliid species occurring close to population centres which, for the reasons mentioned above, may receive an influx of infected carriers, particularly from the highly endemic southern focus. Finally, we review the available data on vector competence of northern Venezuelan populations of *S. metallicum s.l.* and argue that the variable rates of larval success thus far reported are more the result of the operation of density-dependent regulatory processes (Basáñez *et al*. 1995), than the effect of the temperature conditions under which experimentally infected flies have been maintained (Takaoka *et al*. 1984*b*).

MATERIALS AND METHODS

Study area and carriers of O. volvulus

The work was conducted during August of 1985 in the village of Carrasposo (10° 10′ 06″ N, 64° 30′ 30″ W), Anzoátegui State (north-oriental focus), and during September of 1985 in Culebra and Alto-Carinagua, located near Puerto Ayacucho, capital of the Amazonas State (5° 35′ 00″ N, 67° 41′ 49″ W), approximately 500 km from the southern focus (Fig. 1). Two carriers of *O. volvulus* participated in the study. The former was from Caratal, 500 m from Carrasposo (Subject I, a 40-year-old male plantation worker). He had never been elsewhere and was, therefore, considered to be the carrier of the northern parasite population. The latter (Subject II, a 26-year-old Ye’kuana school teacher) had resided for 5–7 years in the Yanomami village of Coyowë-theri (2° 25′ 03″ N, 64° 17′ 57″ W), a highly endemic community of the Amazonian focus where he

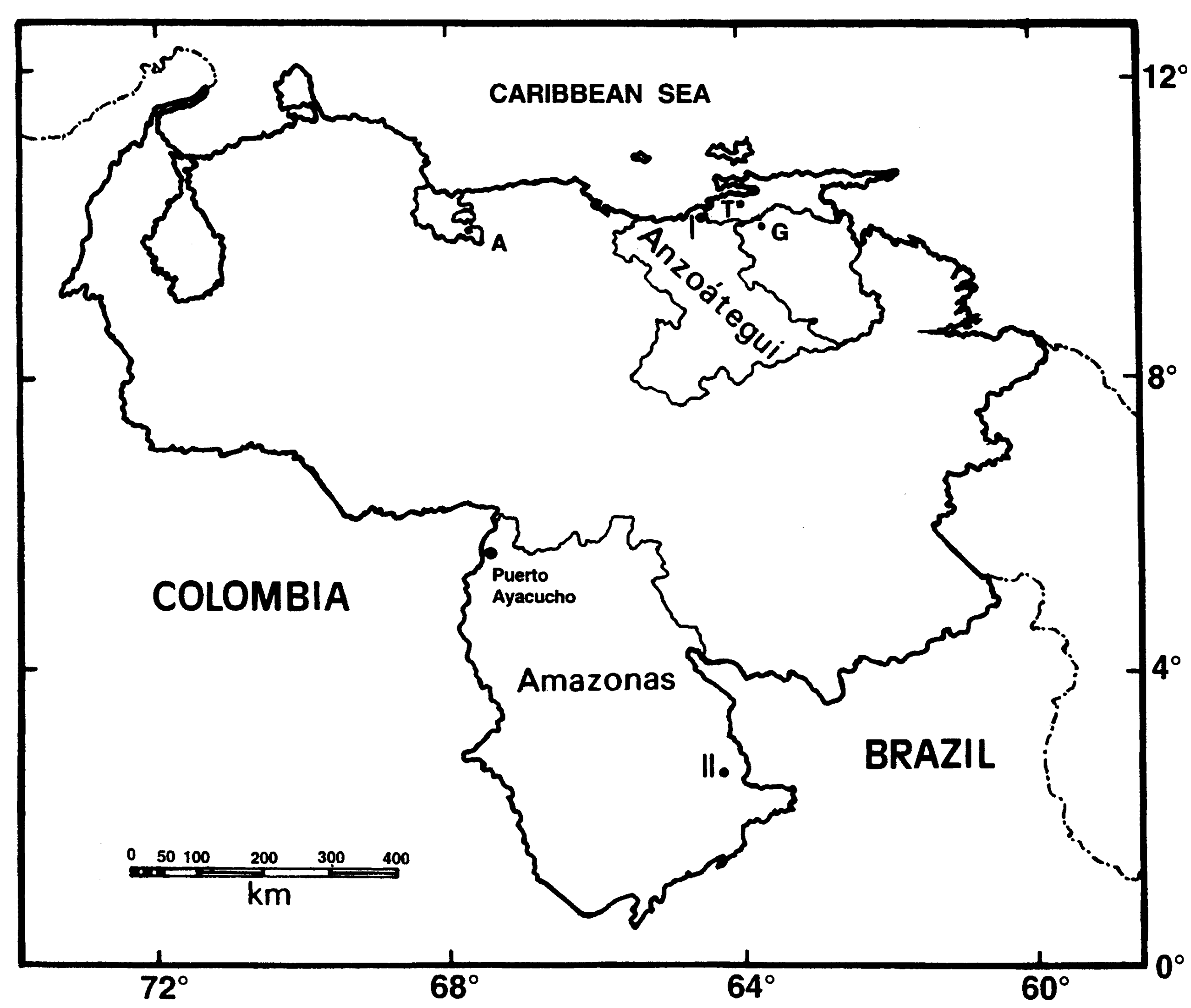


Fig. 1. Map of Venezuela showing the localities where onchocerciasis was acquired by the study participants: **I**, Carrasposo in Anzoátegui State (north-eastern focus, corresponding to subject I), and **II**, Coyowë-theri in Amazonas State (southern focus, corresponding to subject II). Fly-feeding experiments in Amazonas took place in Puerto Ayacucho (outside the endemic area). The map also shows the situation of the localities listed in Table 7 for the analysis of vector competence in Venezuelan populations of *Simulium metallicum s.l.*: **A**, Altamira (south of Lake Valencia), Carabobo State, north-central focus (Duke, 1970; Grillet *et al.* 1994; **G**, Guanaguana (Takaoka *et al.* 1984*b*) and Río Chiquito (Takaoka *et al.* 1986*b*), Monagas State, and **T**, Trincheras (Grillet *et al.* 1994), Sucre State, north-eastern focus.

contracted the infection and became a carrier of the southern parasite population. Table 1 shows their microfilarial loads (mf/mg) in iliac crest and calf obtained by taking, immediately before the fly-feeding experiments, skin biopsies in each of these body regions (2 on the right and 2 on the left hand side) with a Holth sclerocorneal punch followed by incubation of the snips for 24 h in buffered saline solution (Yarzábal *et al.* 1983). The mean microfilarial density (MFD, calculated according to Brandling-Bennett *et al.* 1981) and the prevalence of mf infection in the population of Caratal/Carrasposo were 1·43 mf/mg and 31·4% (11/35), determined at the time of the study, and of 45·12 mf/mg and 84·4% (54/64) in Coyowë-theri (Basáñez & Yarzábal, 1989). Informed consent was obtained from all study participants and the project was approved by the regional health directorates of both Anzoátegui and Amazonas States.

Entomological procedures

In the following sections, *S. metallicum s.l.*, *S. exiguum s.l.* and *S. oyapockense s.l.* will be alluded to without explicit reference as to their *sensu lato* status. The member of the *S. metallicum* complex most likely to be involved in onchocerciasis transmission in both Venezuelan north-central and north-eastern foci is cytospecies E (Conn, 1988; Grillet, Barrera & Conn, 1995). The identity of the cytotypes of *S. exiguum* and *S. oyapockense* occurring in Venezuela is as yet unknown.

At Carrasposo, wild *S. metallicum* flies were individually fed to satiation on the iliac region of

Table 1. Mean *Onchocerca volvulus* microfilarial load (mf/mg) in the skin of subject I (carrier of the north-eastern parasite population) and subject II (carrier of the Amazonian parasite) for experimental infection of 3 simuliid species in northern and southern Venezuela

	Subject I (North-eastern focus)		Subject II (Amazonian focus)	
	Lower back (Ia)	Legs (Ib)	Lower back (IIa)	Legs (IIb)
Positive snips/total	2/4	3/4	4/4	4/4
GM*	1·41	0·94	111·97	10·69
(range)	(0·0–4·1)	(0·0–1·6)	(88·1–155·8)	(3·3–23·1)

* GM, geometric mean microfilarial skin density including positive and negative snips calculated in the same fashion as for the flies (see text).

Subject I and on the calves of Subject II in an attempt to make microfilarial intakes more comparable and avoid possible high fly mortality resulting from the heavy parasite uptakes expected from the iliac region of Subject II (Omar & Garms, 1977). Ten females of *S. exiguum* from this locality were also fed on Subject I and dissected from day 0 through day 10 after the feed. (The very low man-biting density of *S. exiguum* during the sampling period at Carrasposo prevented a fuller evaluation of its compatibility with the parasite populations under study.) At Culebra and Alto Carinagua (Puerto Ayacucho), wild flies of *S. oyapockense* and *S. exiguum* were fully engorged on the iliac zone of Subject I and on both legs and lower back of Subject II in an attempt to maximize the probability of infection of *S. oyapockense*. In contrast with *S. metallicum* and *S. exiguum*, *S. oyapockense* possesses a well-developed cibarial armature that destroys an important but variable proportion of ingested microfilariae (Shelley *et al*. 1987). The fraction of parasites injured by 'armed' simuliids has been shown to be dependent on the density of the intake, decreasing as mf load increases (Basáñez *et al*. 1995). The total no. of *S. metallicum*, *S. exiguum*, and *S. oyapockense* engorged on each carrier were, respectively, 133, 123 and 182 (Subject I), and 126, 205, and 320 (Subject II), collected over a period of 3–4 days so as to protect the subjects from receiving a higher no. of bites than they would normally be exposed to (average daily biting rates per person were 116 for *S. metallicum* at Carrasposo, 196 for *S. exiguum* and 204 for *S. oyapockense* at Culebra, and 384 for *S. exiguum* and 200 for *S. oyapockense* at Alto Carinagua). Flies were collected in individual tubes positioned early on during the feed above lodged insects in order to avoid escape of fully gorged flies.

A group of about 25–35 flies of each species, body region, subject, and locality, was killed between 0 and 12 h post-engorgement (p.e.) to assess microfilarial intake, damage by the cibarial teeth if present, and migration to the thoracic muscles of the flies. Abdomens were severed and 2 smears on each slide prepared with the bloodmeal and the rest of the abdomen. Once dry, the slides were fixed with methanol in the field and stained with 4% methylene blue in 3% acetic acid in the laboratory (Omar & Garms, 1977). A second group of flies was dissected at 24 h p.e. to evaluate larval establishment in the thorax (L1 larvae). Finally, a third group of flies was kept in captivity according to methods described elsewhere (Basáñez *et al*. 1988), fed on sugar solution with antibiotics, and maintained for 6–10 days p.e. to ascertain larval maturation up to the infective stage (L3 larvae) in the surviving flies. In Carrasposo, flies were maintained at a temperature varying between 22 and 32 °C; in Puerto Ayacucho, flies were kept between 25 and 35 °C in the Simuliidae Laboratory of CAICET. With the exception of the abdomens of the flies dissected at 0–12 h p.e. the remaining insects or insect sections were all fixed in 80% ethanol before their staining with Mayer's haemalum and dissection in glycerine (Nelson, 1958). The following morphobiometrical features of L3 larvae were recorded and compared among *Onchocerca–Simulium* combinations: total length and maximum width; position of and width at the nerve ring; tail length, tail width, and caudal index, the latter defined as the ratio between tail length and tail width measured at the anal pore (Nelson & Pester, 1962; Porter & Collins, 1984).

Natural infection was assessed in a sample of 166 *S. metallicum* collected at the same time as the engorged flies in Carrasposo. Since only 1 insect was infected with an early L1 larva, it is henceforth considered that the parasites found in the experimentally infected flies developed from the feed in question. Since Puerto Ayacucho is not endemic for human onchocerciasis, the natural infection of *S. exiguum* and *S. oyapockense* was not investigated.

Data analysis

The proportions of flies with ingested mf, thoracic larvae, and developing and/or infective larvae among *S. metallicum*, *S. exiguum*, and *S. oyapockense* fed on each subject and body regions, were compared using the chi-square test with Yates' correction in $2 \times c$

contingency tables, where *c* refers to the number of species being compared (Pagano & Gauvreau, 1993). Fisher exact test (two-tailed) was applied to 2×2 contigency tables when necessary (Kirkwood, 1988). Because parasite distributions among flies were strongly overdispersed (data not shown), the numbers of larvae per fly among simuliid species were compared using the Kruskal–Wallis test (the non-parametric equivalent of the one-way analysis of variance), correcting the test criterion, *H*, for tied scores (Elliot, 1977). The intensity of infection among flies is reported as the geometric mean (GM) no, of larvae/fly, obtained as follows (Duke, Moore & De León, 1968),

$$GM = \left[\text{anti log} \left(\frac{\sum_{i=1}^{i=j} \log (x_i)}{j} \right) \right] \frac{j}{n},$$

where x_i is the number of larvae in the *i*th of *j* positive flies among a total of *n* flies examined. Mean infective larval measurements were compared using one-way analysis of variance. When results were significant pairs of means were compared by *t*-tests using the multiple procedure of Bonferroni, in which the significance level that must be applied to each individual comparison depends on the predetermined overall level of significance and the number of tests being conducted (Pagano & Gauvreau, 1993). Since the ratio of 2 random normal variates is itself not normally distributed, comparisons of caudal indices were carried out using the Kruskal–Wallis test (Siegel, 1956).

Vector competence of S. metallicum *cytospecies* E *in northern Venezuela*

The term vector competence encapsulates those factors affecting pathogen uptake by, development within, and output from the vector host (Spielman & James, 1990). Analysis of data from this work as well as from Duke (1970), Takaoka *et al*. (1984*b*, 1986a, *b*), Grillet (1993) and Grillet *et al*. (1994) focused, therefore, on the following variables: mean microfilarial skin load/mg, mean microfilarial intake/fly, and proportion of flies with ingested mf (uptake); mean no. of L3/fly and proportion of flies with L3 larvae (development), and parasite yield (output) defined as the proportion of L3/fly developing in relation to mean mf load (Pichon, Perrault & Laigret, 1974).

Dermal intensity of infection and microfilarial intake. In all published data sets, with the exception of that presented by Duke (1970), Holth-type sclerocorneal punches were used to obtain skin snips (ss). When dermal loads were reported as mean no. of mf/ss, the figures were divided by 0·67, the average weight in milligrams of a snip taken with a Holth punch in Neotropical onchocerciasis surveys (Tidwell *et al*. 1980; Collins *et al*. 1980), to obtain the mean no. mf/mg. This differs from the mean weight of 2·8 mg/snip reported for west African settings (Prost & Prod'hon, 1978). Linear regression methods with least squares estimation of the parameters were used to explore the relationship between mean mf intake/fly, *m*, and mean mf skin load/mg, *M*, after taking the logarithm of both variables, a variance-stabilizing transformation (Basáñez *et al*. 1994). The original model is therefore $m = \alpha M^{\beta}$. When the expression $\text{Log}(m) = \text{Log}\,\alpha + \beta\,\text{Log}(M)$ was fitted to the data, the intercept, Log α, proved to be indistinguishable from zero (corresponding to $\alpha = 1$); therefore the function $\text{Log}(m) = \beta\,\text{Log}(M)$ was used. The null hypothesis, i.e. that there is proportionality between the original variables, corresponds to $\beta = 1$; $\beta \neq 1$ indicates non-linearity (operation of positive or negative feed-back processes if $\beta > 1$ or $\beta < 1$, respectively).

Proportion of flies with ingested mf and mean microfilarial intake. When the frequency distribution of the no. mf/fly is overdispersed, the negative binomial provides an adequate model (Basáñez *et al*. 1994). In consequence, the relationship between *p*, the proportion of flies with mf in their bloodmeals and *m*, the mean mf intake, was explored by fitting the expression $p = 1 - (1 + m/k(m))^{-k(m)}$ by maximum likelihood methods, where *k* is the clumping parameter of the negative binomial and *k*(*m*) permits investigating a possible dependency of *k* on the mean (Basáñez *et al*. 1995). Likelihood outputs for different assumptions regarding *k*(*m*) were compared using the likelihood ratio statistic, $W = 2[l(\hat{k}_{\text{model 1}}) - l(\hat{k}_{\text{model 2}})]$, which, under the null hypothesis $k_{\text{model 1}} = k_{\text{model 2}}$ has, approximately, a chi-squared distribution with degrees of freedom (D.F.) equal to the difference between the number of parameters estimated by each of the models under comparison (Cox & Oakes, 1984; Clayton & Hills, 1993).

Proportion of L3 larvae developing from a given mf load. For each of the data points available the parasite yield (*PY*) was calculated dividing *L*, the mean no. L3/fly in flies surviving the extrinsic incubation period, by *M* or *m*, the mean no. mf/mg or/fly, and the relationship was explored between *PY* and increasing mf intensity. $PY = L/M$ was finally selected because Grillet *et al*. (1994) reported skin density but not mf intake. The model $PY = \delta/(1 + \gamma M)$ was fitted by non-linear regression methods. Because δ proved to be statistically indistinguishable from 1, the simpler function $PY = 1/(1 + \gamma M)$ was used. The null hypothesis of proportionality corresponds to $\gamma = 0$, indicating that *PY* remains constant (and equal to unity) regardless of mf density; $\gamma > 0$ would describe that *PY* decreases with intensity of infection, i.e. that there is

Table 2. Intake and thoracic establishment of *Onchocerca volvulus* microfilariae from the Venezuelan north-eastern (I) and Amazonian (II) foci in *Simulium metallicum s.l.*, *Simulium exiguum s.l.* and *Simulium oyapockense s.l.*

(The statistical tests comparing the numbers of larvae/fly among fly species are not performed on the geometric means but on the raw counts (see Appendix). The GM values are shown here to summarize the data in a format comparable with published accounts; **a**, indicates iliac region; **b**, denotes legs. Fractions and means followed by the same letter are not significantly different.)

Blackfly species… (cibarium)		*S. metallicum s.l.* (unarmed)	*S. exiguum s.l.* (unarmed)	*S. oyapockense s.l.* (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
0–12 h p.e.				
No. of flies with ingested mf/total dissected (%)	Ia	14/24^{c} (58·3)	17/23^{c} (73·9)	5/33^{d} (15·2)
	IIa	—	25/25^{c} (100·0)	29/30^{c} (96·7)
	IIb	21/25^{c} (84·0)	29/36^{c} (80·6)	17/26^{c} (65·3)
GM no. of ingested mf/fly	Ia	2·13^{c}	2·43^{c}	0·20^{d}
	IIa	—	184·53^{c}	165·48^{c}
	IIb	10·86^{c}	12·90^{c}	7·21^{c}
No. of flies with thoracic mf/total dissected (%)	Ia	3/24^{c} (12·5)	3/23^{c} (13·0)	1/33^{c} (3·0)
	IIa	—	23/25^{c} (92·0)	14/30^{d} (46·7)
	IIb	15/25^{c} (60·0)	20/36^{c} (55·6)	1/26^{c} (3·9)
GM no. of thoracic mf/fly	Ia	0·24^{c}	0·38^{c}	0·03^{c}
	IIa	—	18·14^{c}	2·39^{d}
	IIb	2·02^{c}	2·99^{c}	0·08^{d}
24 h p.e.				
No. of flies with early L1/total dissected (%)	Ia	18/24^{c} (75·0)	22/33^{c} (66·7)	0/42^{d} (0·0)
	IIa	—	4/4^{c} (100·0)	18/22^{c} (81·8)
	IIb	13/22^{c} (59·1)	28/43^{c} (65·1)	2/46^{d} (4·4)
GM no. of early L1/fly	Ia	2·88^{c}	1·98^{c}	0·00^{d}
	IIa	—	13·96^{c}	2·70^{c}
	IIb	3·29^{c}	2·68^{c}	0·12^{d}
6–10 days p.e.				
No. of flies with larvae (all stages)/total dissected (%)	Ia	47/80^{c} (58·8)	10/16^{c} (62·5)	1/51^{d} (2·0)
	IIa	—	8/8^{c} (100·0)	32/64^{d} (50·0)
	IIb	47/73^{c} (61·6)	21/34^{c} (61·8)	3/71^{d} (4·2)
GM no. of larvae/fly	Ia	1·82^{c}	2·02^{c}	0·04^{d}
	IIa	—	4·50^{c}	1·56^{d}
	IIb	2·17^{c}	2·16^{c}	0·06^{d}

density-dependent limitation of larval establishment/development within *S. metallicum*. Proportionality or limitation are the 2 alternative patterns described for the relationship between mf input and L3 output in 'unarmed' vectors (Basáñez *et al*. 1995). For all the tests performed in this work the level of significance was set to 0·05 and they were carried out using Statistica™ (StatSoft Inc., Tulsa, OK).

RESULTS

Intake, establishment and development of O. volvulus *from northern and southern Venezuela in sympatric and allopatric species of* Simulium

In this work, only the combination *S. metallicum–O. volvulus* from the North was truly sympatric. Tables 2 and 3 summarize, for *S. metallicum* from Carrasposo (north-eastern Venezuela), and *S. exiguum* and *S. oyapockense* from Puerto Ayacucho (Amazonas region), the results of the feeding experiments conducted on Subjects I and II (*a* stands for iliac and *b* for legs). Table 2 shows that, when fed on the same body regions, the 3 simuliid species did not generally differ in their microfilarial intakes (with the exception of *S. oyapockense* fed on Ia). The values of χ^2 (chi-square tests on the proportions of flies infected), *H* (Kruskal–Wallis criterion for the numbers of parasites/fly), and associated *P* values and D.F. are listed in Appendix A. Although *S. exiguum* ingested comparatively more mf than either *S. metallicum* or *S. oyapockense*, this difference was not significant for either IIa or IIb. In contrast, when fed on Subject Ia, the fraction of infected *S. oyapockense* flies, and

Table 3. Development of *Onchocerca volvulus* microfilariae from the Venezuelan north-eastern (I) and Amazonian (II) foci in *Simulium metallicum s.l.*, *Simulium exiguum s.l.* and *Simulium oyapockense s.l.*

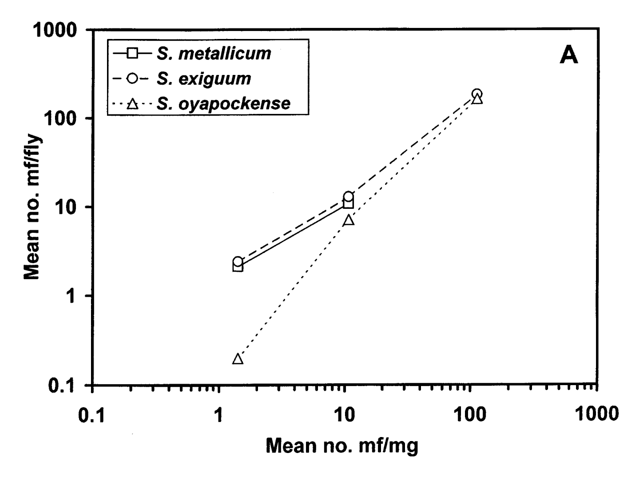
(See footnotes to Table 2.)

Blackfly species... (cibarium)		*S. metallicum s.l.* (unarmed)	*S. exiguum s.l.* (unarmed)	*S. oyapockense s.l.* (armed)
Locality...	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
6–10 days p.e.				
No. of flies with L1–L2/ total dissected (%)	Ia	33/80[c] (41·3)	10/16[c] (62·5)	1·51[d] (2·0)
	IIa	—	8/8[c] (100·0)	5/64[d] (7·7)
	IIb	45/73[c] (61·6)	21/34[c] (61·8)	0/71[d] (0·0)
GM no. of L1–L2 larvae/fly	Ia	0·97[c]	2·02[c]	0·04[d]
	IIa	—	4·50[c]	0·13[d]
	IIb	2·13[c]	2·16[c]	0·00[d]
No. of flies with L3/ total dissected (%)	Ia	21/80[c] (26·3)	0/16[d] (0·0)	0/51[d] (0·0)
	IIa	—	0/8[c] (0·0)	29/64[d] (45·3)
	IIb	4/73[c] (5·5)	0/34[c] (0·0)	3/71[d] (4·2)
GM no. of L3 larvae/fly	Ia	0·95[c]	0·0[d]	0·00[d]
	IIa	—	0·0[c]	1·49[d]
	IIb	0·11[c]	0·0[c]	0·06[c]
Parasite yield (%)	Ia	44·6	0·0	0·0
	IIa	—	0·0	1·1
	IIb	1·0	0·0	0·8

the number of parasites ingested/fly were lower than those of *S. metallicum* or *S. exiguum* (see also Fig. 2A). *S. metallicum* and *S. exiguum* also resembled each other regarding microfilarial migration to the thorax measured at 0–12 h p.e. (Appendix B) and larval establishment measured both at 24 h p.e. (early L1 stages: Appendix C) and at 6–10 days p.e. (all larval stages: Appendix D). In contrast, *S. oyapockense* exhibited, in most cases, a significantly lower proportion of flies with thoracic larvae and fewer numbers of larvae/fly than either *S. metallicum* or *S. exiguum*. *S. oyapockense* destroyed a high proportion of the ingested parasites; the fraction damaged appeared to decrease as the mean intake increased (Table 4; $\chi^2 = 1\ 0{\cdot}59$ and $P = 0{\cdot}001$ with 1 D.F. for the comparison IIa–IIb). The non-significant difference between Ia and II ($\chi^2 = 0{\cdot}99$ and $P = 0{\cdot}32$ for the comparison Ia–IIa; $\chi^2 = 0{\cdot}19$ and $P = 0{\cdot}67$ for Ia–IIb, both with 1 D.F.) is probably due to the small sample size in case of the former. Fig. 2B depicts the increasing proportion of undamaged mf as a function of mf skin density. This effect is reflected in the numbers of mf available for migration to and establishment/development in the thorax of the flies, since in *S. oyapockense* both the proportion of flies with larvae and the numbers of larvae per fly were highest for those flies fed on IIa (Table 2).

Regarding larval development (Table 3), there were no significant differences between *S. metallicum* and *S. exiguum* concerning the fraction of flies with and the numbers of developing L1–L2 stages (Appendix E), although in southern *S. exiguum* larval maturation did not proceed beyond the L1 stage. The larvae observed during 6–10 days p.e. in this simuliid population were typically stunted, malformed, and with disintegrating internal structures. Some degree of delayed larval development also took place in *S. metallicum* as attested by the similarity between this species and *S. exiguum* in relation to the fraction of flies with developing larvae and the larval load per fly. In contrast, parasite maturation to the L3 stage was more synchronous in *S. oyapockense* infected with southern *O. volvulus*. Despite this, the proportion of larvae reaching the infective stage per ingested mf was 45% for the sympatric combination *S. metallicum*–northern *O. volvulus* in comparison to 1% for the allopatric counterpart. The parasite yield for *S. oyapockense*–southern *O. volvulus* was 1% whereas there was no infective larval output for the heterologous combination.

Table 5 indicates that although there was no difference between the northern and southern parasite populations regarding the proportion of *S. metallicum* flies with cephalic larvae ($L3_H$: Fisher exact $P = 0{\cdot}84$), the fraction of infective larvae migrating to the head was 75% in the sympatric compared to 33% in the allopatric combination ($\chi^2 = 6{\cdot}93$ and $P = 0{\cdot}008$, 1 D.F.). When *S. oyapockense* from the Puerto Ayacucho (fed on the carrier of Amazonian *O. volvulus*, both body regions com bined), was compared with *S. metallicum* (fed on both carriers), there was again no difference in the proportion of flies with L3 in the head ($\chi^2 = 2{\cdot}89$ and $P = 0{\cdot}24$, 2 D.F.). The infective stages of the southern parasite did not differ in their ability to migrate to



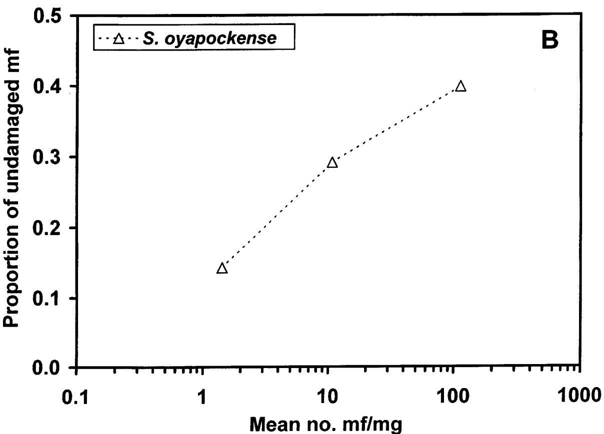


Fig. 2. The relationship, in the *Onchocerca volvulus–Simulium* combinations investigated in this study (Tables 1, 2 and 4), between mean microfilarial skin load and (A) mean microfilarial intake per fly; (B) proportion of mf found undamaged in the bloodmeal of the species with cibarial armature (*S. oyapockense s.l.*).

the cephalic capsule of the flies either in allopatric *S. metallicum* or in Puerto Ayacucho *S. oyapockense* ($\chi^2 = 2{\cdot}04$ and $P = 0{\cdot}15$, 1 D.F.), but did differ from those of the northern parasite in homologous *S. metallicum* ($\chi^2 = 42{\cdot}73$ and $P \ll 0{\cdot}001$, 2 D.F.), indicating that the number of fully infective, cephalic larvae, was higher in the truly sympatric infection.

Table 6 summarizes the morphobiometrical data for the infective larvae harboured by *S. metallicum* and *S. oyapockense* 6–10 days p.e. Although L3 deriving from the heterologous combination southern *O. volvulus*–northern *S. metallicum* were apparently shorter than those developing from the local counterpart, the difference was not significant (t-value $= 1{\cdot}89$, one-tailed $P = 0{\cdot}03$ and 34 D.F. in the Bonferroni multiple comparisons procedure with individual significance level set at $0{\cdot}017 = 0{\cdot}05/3$). However, the L3 obtained from *S. metallicum* were shorter ($t = 2{\cdot}39$, $P = 0{\cdot}01$), and in the case of the sympatric northern combination, wider ($t = 3{\cdot}82$, $P < 0{\cdot}001$) than those harboured by *S. oyapockense* infected with the southern parasite. The distance from the nerve ring to the cephalic end was longer in the southern L3 obtained from this simuliid ($t = 4{\cdot}60$, $P \ll 0{\cdot}001$ when compared with those from the homologous northern combination), but in all remaining features the infective stages were similar. All L3 larvae had 3 caudal papillae and a caudal index of about 2, typical of *O. volvulus* (Duke, 1967; Bain & Chabaud, 1986).

Of the 10 flies of northern *S. exiguum* fed on Subject I, 4 were found uninfected at the time of dissection (0–8 days p.e.); 4 harboured a geometric mean of 10·5 early L1 larvae/fly (12–72 h p.e.); 1 had 9 L1 and 1 L2 on day 9 p.e., and 1 was infective on day 10 p.e. with 1 L3 thoracic larva.

Vector competence of S. metallicum *cytospecies* E *in northern Venezuela*

Table 7 summarizes available data on vector competence of Venezuelan *S. metallicum* as obtained in homologous experimental infections with *O. volvulus* from the north-central and north-eastern foci. The data are arranged in increasing order of skin mf density. Neither the mean no. of L3 per infective fly (2–5) nor the proportion of flies with infective larvae (0·03–0·4) show any systematic variation with temperature of fly maintenance; the consequence, the mean no. of L3 per dissected fly (L) is also rather

Table 4. Proportion of *Onchocerca volvulus* microfilariae from the Venezuelan north-eastern (I) and Amazonian (II) foci damaged by the cibarial armature of *Simulium oyapockense s.l.*

(95% CL are the exact values obtained using the F distribution (Armitage & Berry, 1994).)

Subject	Mean mf intake	Total no. of scored mf	Fraction of damaged mf	95% CL	
				Lower	Upper
Ia	0·2	7	0·857	0·421	0·996
IIb	7·2	237	0·709	0·651	0·767
IIa	165·5	6857	0·602	0·590	0·613

Table 5. Proportion of flies with cephalic L3 larvae among infective flies and proportion of L3 in the head among infective stages in *Simulium metallicum s.l.* and *Simulium oyapockense s.l.* 6–10 days after engorgement on *Onchocerca volvulus* carrier from the Venezuelan north–eastern and Amazonian foci

(Fractions followed by the same letter are not significantly different.)

Blackfly species	Subject I (North-eastern parasite)	Subject II (Amazonian parasite)
S. metallicum s.l. (Carrasposo)		
No. of flies with $L3_H$/total infective flies (%)	19/21[c] (90·5)	3/4[c] (75·0)
No. of L3 Head/total infective larvae (%)	69/92[c] (75·0)	4/12[d] (33·3)
S. oyapockense s.l. (Puerto Ayacucho)		
No. of flies with $L3_H$/total infective flies (%)	0/0	24/32[c] (75·0)
No. of L3 Head/total infective larvae (%)	0/0	77/130[d] (59·2)

Table 6. Mean dimensions ± S.D. (in μm) of infective larvae deriving from homologous and heterologous infections of *Simulium metallicum s.l.* and *Simulium oyapockense s.l.* 6–10 days after engorgement on *Onchocerca volvulus* carriers from the Venezuelan north-eastern and Amazonian foci

Blackfly species	Subject I (North-eastern parasite)	Subject II (Amazonian parasite)
S. metallicum s.l. (Carrasposo)		
n	13	3
Total length	512·9[c] ± 56·9*	453·9[c] ± 66·0
Maximum width	20·9[c] ± 1·7	19·3[c] ± 1·2
Position of nerve ring	61·6[c] ± 4·2	58·7[c] ± 2·8
Width at nerve ring	18·1[c] ± 1·0	18·1[c] ± 1·2
Tail length	33·9[c] ± 3·8	32·1[c] ± 2·8
Tail width	17·0[c] ± 0·3	16·5[c] ± 0·7
Caudal index	2·00[c]	1·95[c]
S. oyapockense s.l. (Puerto Ayacucho)		
n		21
Total length		554·1[d] ± 40·8
Maximum width		19·4[d] ± 0·5
Position of nerve ring		69·4[d] ± 5·3
Width at nerve ring		17·3[c] ± 0·9
Tail length		33·0[c] ± 2·3
Tail width		16·7[c] ± 0·8
Caudal index		1·98[c]

* These measurements compare well with those obtained by Grillet (1993) for sympatric *O. volvulus–S. metallicum* infections in the north-central and north-eastern localities of Altamira (456·2 ± 86·0 to 494·8 ± 65·3 μm) and Trincheras (478·0 ± 42·9 to 521·7 ± 60·7 μm) respectively.

constant. Most of the fly-feeding experiments had been performed during the wet season with the exception of AD and TD from Grillet *et al*. (1994). However, these do not show a consistent difference between the two seasons. Instead, there is an evident declining trend for the parasite yield with intensity of skin infection.

Fig. 3 A depicts the relationship between the log of ingested mf/fly and the log of skin mf/mg. The regression line goes through the origin with slope $\hat{\beta} \pm \text{S.E.}(\hat{\beta}) = 0{\cdot}958 \pm 0{\cdot}103$ ($r = 0{\cdot}87$). Because the 92% C.L. for the regression coefficient (0·706–1·211) included 1, the hypothesis of proportionality between the original variables (mf intake and skin density) was maintained. The relationship between the proportion of flies with ingested mf and mf intake was, on the contrary, strongly non-linear (Fig. 3B), with linear k, i.e. $k(m) = k_0 + k_1 m$ ($k_0 = 0{\cdot}560$, $k_1 = 0{\cdot}042$, $l_L = -125{\cdot}853$) providing a significantly better fit than constant k ($k = 0{\cdot}997$, $l_C = -128.411$),

Table 7. Summary of available data on vector competence of populations of *Simulium metallicum s.l.* (cytospecies E) for *Onchocerca volvulus* in northern Venezuela

(NC, north-central onchocerciasis focus; NE, north-eastern onchocerciasis focus; W, wet season; D, dry season.)

Subject	Mean no. of mf/mg (M)	Mean no. of mf/+ve fly	Flies with mf/total flies dissected	Mean no. of mf/fly (m)	Mean no. of L3/+ve fly	Flies with L3/total flies dissected	Mean no. of L3/fly (L)	Parasite yield		Locality	State (focus)	Month-year (season)	Temperature of maintenance (reference)
								L/M	L/m				
Ia	1·40	3·36	13/23	1·90	3·13	23/80	0·95	0·67	0·45	Carrasposo	Anzoátegui (NE)	Aug-1985 (W)	22–32 °C (This work)
VIII	1·67	3·78	53/72	2·78	1·85	44/136	0·58	0·35	0·21	Altamira	Carabobo (NC)	Oct/Nov-1969 (W)	23–29 °C (Duke, 1970)
—	2·99	4·50	8/20	1·80	2·90	14/111	0·37	0·12	0·21	Guanaguana	Monagas (NE)	Nov-1982 (W)	22–28 °C (Takaoka *et al.* 1984*b*)
IX	5·00	11·00	95/106	9·86	2·41	77/210	0·86	0·17	0·09	Altamira	Carabobo (NC)	Oct/Nov-1969 (W)	23–29 °C (Duke, 1970)
X	11·80	20·30	100/102	19·90	2·37	23/80	0·66	0·06	0·03	Altamira	Carabobo (NC)	Oct/Nov-1969 (W)	23–29 °C (Duke, 1970)
AD	13·00	—	—	—	3·18	17/160	0·34	0·03	—	Altamira	Carababo (NC)	Mar-1990 (D)	27 °C (Grillet *et al.* 1994)
AW	13·00	—	—	—	1·77	13/138	0·17	0·01	—	Altamira	Carabobo (NC)	Sep-1990 (W)	27 °C (Grillet *et al.* 1994)
TD	13·50	—	—	—	5·00	2/78	0·13	0·01	—	Trincheras	Sucre (NE)	Feb-1990 (D)	27 °C (Grillet *et al.* 1994)
TW	13·50	—	—	—	2·93	14/155	0·26	0·02	—	Trincheras	Sucre (NE)	Aug-1990 (W)	27 °C (Grillet *et al.* 1994)
FA Rodríguez	19·40	9·00	13/16	7·31	3·00	7/45	0·45	0·02	0·06	Río Chiquito	Monagas (NE)	Aug-1984 (W)	20–24 °C (Takaoka *et al.* 1986*b*)
FA Rodríguez	58·21	48·00	20/20	48·00	3·00	2/7	0·86	0·01	0·02	Río Chiquito	Monagas (NE)	Aug-1984 (W)	20–24 °C (Takaoka *et al.* 1986*b*)

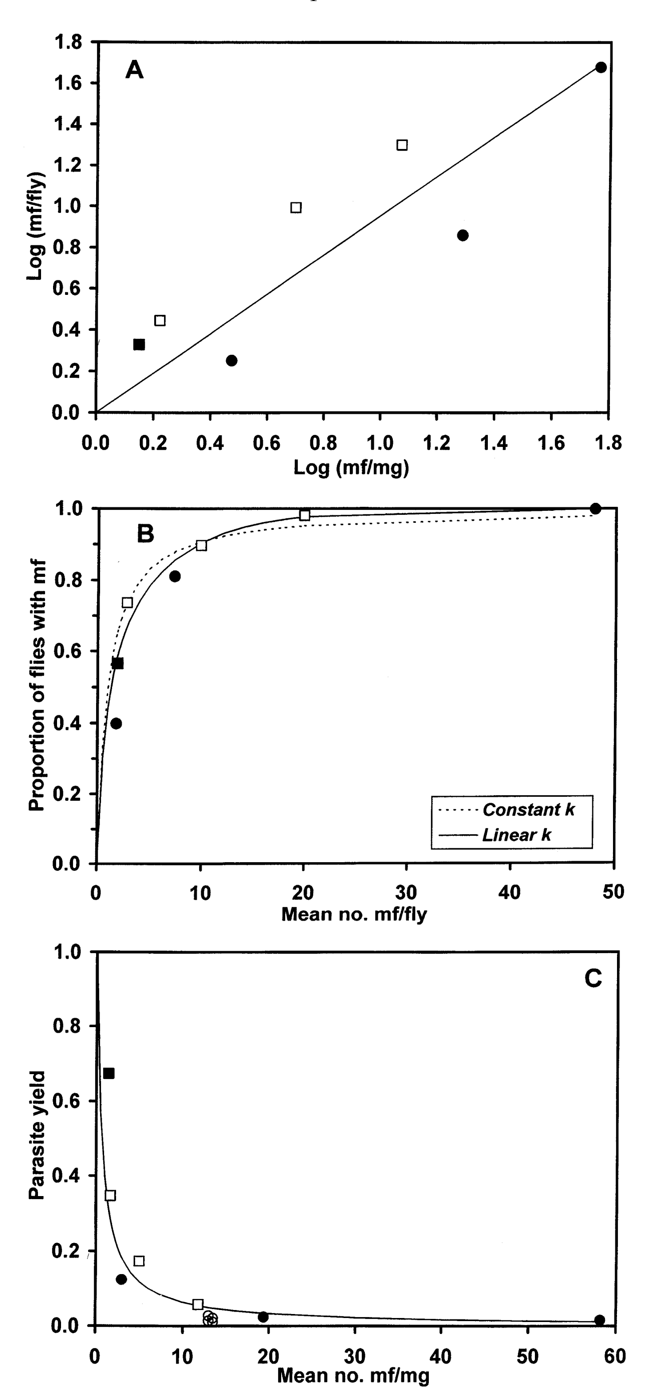


Fig. 3. The relationship, in the sympatric *Onchocerca volvulus–S. metallicum* combinations investigated in Venezuela (Table 7), between mean microfilarial skin load and (A) mean microfilarial intake per fly. The fitted line is the regression function Log $(m) = \beta$ Log (M) where m = mean no. of mf/fly and M = mean no. of mf/mg; (B) proportion, p, of flies with ingested mf, m. The lines correspond to the expression $p = 1-(1+m/k(m))^{-k(m)}$ with negative binomial parameter k fitted as a constant (––––), or as a linear function of the mean (——). (C) Parasite yield, PY, calculated as the proportion of mf/fly developing to the infective stage. The fitted line is the monotonically decreasing function $PY = (1+\gamma M)^{-1}$ (see text). Markers are data points from: (■) this work; (□) Duke (1970); (●) Takaoka *et al.* (1984*b*, 1986*b*), and (○) Grillet (1993) and Grillet *et al.* (1994).

with $W = 5{\cdot}12$, and $P = 0{\cdot}024$. Finally, parasite yield was a monotonically decreasing function of skin load (Fig. 3C), with $\hat{\gamma} \pm$ S.E.$(\hat{\gamma})$ (parameter estimate of the severity of density-dependence) equal to $1{\cdot}515 \pm 0{\cdot}285$ (95% C.L. = 0·881–2·150). This indicates a strong limitation of parasite development to the infective stage in *S. metallicum*, with the non-linear model explaining 77·3% of the observed variation ($r = 0{\cdot}88$).

DISCUSSION

Because we did not attempt to tease apart blackfly susceptibility and onchocercal infectivity, we use the term compatibility (Morand, Manning & Woolhouse, 1996) to refer to infective larval output in the flies, in terms of both prevalence and intensity of infection. A greater compatibility between local *O. volvulus–Simulium* populations was found in the case of *S. metallicum* when compared with the allopatric combination. Due to the phenomenon of limitation herein described for *S. metallicum*, and with the parameter values estimated, the parasite yield (L/M) for a skin load of 10·7 mf/mg (corresponding to the legs of the carrier of the southern parasite) would have been expected to vary between 4 and 10%. In contrast, only 1% of these mf reached the infective stage. From the data it is clear that this difference cannot be ascribed to defective microfilarial migration or early establishment in the thoracic muscles but rather to mechanisms acting on larval moult and/or development to the L3 stage. Migration to the head of the flies, but not total larval length, was also reduced in the heterologous infection.

S. metallicum for the northern focus and *S. exiguum* from Puerto Ayacucho did not differ in their microfilarial uptake or in the ability of the ingested mf to invade their thoracic muscles; neither did they differ in their pre-infective larval loads or in the proportion of flies with L1–L2 on days 6–10 p.e. This supports previous reports of delayed larval development and certain degree of asynchrony in both *S. metallicum* (Collins, 1979; Tidwell *et al.* 1980; Grillet *et al.* 1994) and *S. exiguum* (Shelley & Arzube, 1985; Tidwell *et al.* 1980; Collins *et al.* 1995). However, in contrast to *S. metallicum*, no larval development proceeded beyond the L1 stage in this middle Orinoco population of *S. exiguum*. Although very few north-eastern *S. exiguum* were infected in this work, 1 of 10 flies fed on Subject I harboured one L3 larva on day 10 p.e. confirming earlier findings on sympatric infections (Duke, 1970). The *S. exiguum* complex has an ample distribution is South America comprising a number of sibling cytospecies and related cytotypes (Shelley, 1988; Charabous, Shelley & Arzube, 1993), which vary widely in their vector efficiency (Shelley *et al.* 1997). It is likely that S. exiguum from north-eastern Venezuela belongs to a different cytotype from that

of the population biting at Puerto Ayacucho, and that these are themselves different from the populations present in Ecuadorian/Colombian foci.

Due to the presence of a cibarial armature, *S. oyapockense* exhibited reduced rates of microfilarial migration and establishment in the thorax of the flies, particularly when microfilarial intakes were low (Ia and IIb). There was no development of the northern parasite to the infective stage in this simuliid. However, southern *O. volvulus* developed synchronously in *S. oyapockense* from Puerto Ayacucho and parasite yields (about 1 %) were compatible with those observed in sympatric *S. ochraceum* (armed)–*O. volvulus* from Guatemala (Collins *et al.* 1977). An increasing proportion of unscathed mf with heavier intakes is also in agreement with available observations for the Central American species (Basáñez *et al.* 1995). The body lengths of *O. volvulus* L3 larvae obtained from *S. metallicum* were shorter than those from *S. oyapockense*. A similar trend has been observed when infective larvae recovered from *S. metallicum* were compared to those from *S. ochraceum* in Guatemala (Collins, 1979; Ito, Tanaka & Ochoa, 1980).

Microfilarial uptake was found to be roughly proportional to skin density in *S. metallicum*, *S. exiguum* and *S. oyapockense* as already demonstrated for savanna *S. damnosum*, *S. guianense* and *S. ochraceum* (Basáñez *et al.* 1994). Based on our analyses, we find little evidence in support of microfilarial concentration as it has been defined in other *Onchocerca*–*Simulium* studies i.e. higher numbers of ingested mf than would be expected from skin loads (De León & Duke, 1966) or saturation i.e. mf intakes levelling-off with increasing skin load (Duke, 1962). The problems associated with comparing no. mf/fly with no. mf/mg instead of no. mf per volume of blood in both vector and human hosts have been discussed by Basáñez *et al.* (1994). Density-dependent regulation, however, does affect parasite survival/development in the combinations explored here; in *S. metallicum* from northern Venezuela (cytospecies E), a decreasing rate of larval success with mf density indicates the operation of limitation, probably acting through delayed development rather than through mechanisms associated with the peritrophic matrix, which is thin and slow to form in *S. metallicum* (Lewis & Garnham, 1959; Omar & Garms, 1977). Thus, variable outcome of sympatric *O. volvulus*–*S. metallicum* infections may be more the product of processes regulating parasite abundance than of variable maintenance conditions (Takaoka *et al.* 1984*b*). Conversely, density-dependent damage of ingested mf by *S. oyapockense* could result in initial facilitation as occurs in *S. ochraceum* (Basáñez *et al.* 1995; Collins *et al.* 1995). This conjecture is currently being tested with feeding experiments in the Amazonian focus of southern Venezuela.

A value of the negative binomial parameter *k* which increases with the mean parasite load has been interpreted as an indication of decreasing aggregation suggestive of the operation of density dependent processes (Guyatt *et al.* 1990). In *S. metallicum*, mortality induced by heavy mf intakes, of the type described for unarmed *S. damnosum* and *S. guianense* (Basáñez *et al.* 1996), has been demonstrated by following the survival of fly cohorts fed on carriers with varying intensity of infection (De León & Duke 1966). Among freshly engorged *S. metallicum*, a better fit to the relationship between proportion of flies with ingested mf and mean mf intake was indeed obtained with a linear rather than a constant *k*. A frequently made interpretation of this result is to ascribe it to early action of parasite-induced vector mortality since this mechanism would produce decreasing overdispersion by truncating the tails of distributions as mf intake increases. However, it is possible to demonstrate theoretically that a reduction of vector survival due to increasing parasite acquisition can be accompanied by a constant value of the parameter *k* (Dietz, 1976 and personal communication).

The population of *S. exiguum* investigated in the present work seems to be refractory to the maturation of both northern and southern Venezuelan *O. volvulus*, larval development ceasing at the L1 stage for either parasite population without any evidence of melanotic encapsulation. A similar phenomenon has been reported when forest members of *S. damnosum s.l.* are intra-thoracically infected with *O. lienalis* or *O. gutturosa*, or when Liberian (forest) *S. sanctipauli* is injected with savanna *O. volvulus* mf (Ham & Garms, 1986, 1987). Only *per os* infections were conducted here, and the mechanisms involved in the observed resistance of *S. exiguum* do not appear to affect mf intake or ability to migrate out of the bloodmeal and into the haemocoele of the flies, contrasting with results for other filaria–vector combinations (Beernsten *et al.* 1995). More likely, they seem to be driven by tissue-associated factors operating in the thoracic muscles (Wattam & Christensen, 1992; Ham *et al.* 1994).

The existence of locally adapted *Onchocerca*–*Simulium* complexes in west Africa and Neotropical regions has been proposed to be indicative of considerable evolutionary divergence between *O. volvulus* endemic in these 2 geographical areas (Duke, 1970, 1981). More recent work has, however, demonstrated that Central and South American parasite populations are closer to West African savanna isolates than they are to each other (Zimmerman *et al.* 1994). It has also been suggested that hybridization may be taking place between forest and savanna strains in those areas of West Africa where both co-occur (Toé *et al.* 1997). The northern and southern Venezuelan *O. volvulus* populations are geographically isolated by the central plains

('Llanos'), and our results indicate strong local adaptation; however, this may not be accompanied by extensive parasite divergence. In fact, local adaptation of parasites to their hosts does not necessarily imply reproductive isolation or significant reduction of gene flow among parasite populations. Strong local selection in the absence of parasite diversification would be expected for any obligatory parasite that is dispersing among markedly differentiated host populations (Dybdahl & Lively, 1996).

In conclusion, geographical isolation, strong local parasite–vector adaptation, density-dependent limitation and high mortality of *S. metallicum* when fed on heavy mf loads typical of Amazonian focus, destruction of ingested mf by *S. oyapockense* when infected with low numbers of mf characteristic of the northern foci, and refractoriness of southern *S. exiguum* populations, all make it unlikely that human onchocerciasis would spread readily between the northern and southern foci of Venezuela. However, the Amazonian focus of human onchocerciasis could extend its range outside its present distribution (Upper Orinoco region) as middle Orinoco populations of *S. oyapockense* bite humans in large numbers (we have recorded daily biting rates > 1000 in other localities), are intrinsically susceptible to parasite development particularly when feeding on high microfilarial densities, and would suffer reduced rates of parasite-induced mortality due to the protection afforded by the cibarial armature.

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Appendix A. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* microfilarial intake among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
0–12 h p.e.				
Proportion of	Ia	χ^2_{me} = 0·67, P=0·41, 1 D.F.	χ^2_{mo} = 9·80, P = 0·002, 1 D.F.	χ^2_{eo}=17·23, P<0·001, 1 D.F.
flies with	IIa			χ^2_{eo}=0·85, P=0·36, 1 D.F.
ingested mf	IIb		χ^2_{meo}=2·93, P=0·23, 2 D.F.	
No. of	Ia	H_{me}=0·57, P=0·45, 1 D.F.	H_{mo}=14·29, P<0·001, 1 D.F.	H_{eo}=22·59, P<0·001, 1 D.F.
ingested	IIa			H_{eo}=0·16, P=0·69, 1 D.F.
mf/fly	IIb		H_{meo}=3·25, P=0·20, 2 D.F.	

a, Iliac region; b, legs; *me*, compares *S. metallicum* with *S. exiguum*; *mo*, compares *S. metallicum* with *S. oyapockense*; *eo*, compares *S. exiguum* with *S. oyapockense*; *meo*, compares *S. metallicum* with *S. exiguum* and *S. oyapockense*.

Appendix B. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* microfilarial migration to the thorax among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus. Footnotes as in Appendix A.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
0–12 h p.e.				
Proportion of	Ia		$\chi^2_{me}=2{\cdot}31$, $P=0{\cdot}32$, 2 D.F.	
flies with	IIa			$\chi^2_{eo}=10{\cdot}75$, $P=0{\cdot}001$, 1 D.F.
thoracic mf	IIb	$\chi^2_{me}=0{\cdot}007$, $P=0{\cdot}93$, 1 D.F.	$\chi^2_{mo}=16{\cdot}15$, $P<0{\cdot}001$, 1 D.F.	$\chi^2_{eo}=15{\cdot}79$, $P<0{\cdot}001$, 1 D.F.
No. of	Ia		$H_{meo}=2{\cdot}37$, $P=0{\cdot}31$, 2 D.F.	
thoracic	IIa			$H_{eo}=23{\cdot}24$, $P\ll 0{\cdot}001$, 1 D.F.
mf/fly	IIb	$H_{me}=0{\cdot}18$, $P=0{\cdot}67$, 1 D.F.	$H_{mo}=16{\cdot}22$, $P<0{\cdot}001$, 1 D.F.	$H_{eo}=17{\cdot}55$, $P<0{\cdot}001$, 1 D.F.

Appendix C. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* larval establishment (early L1) among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus. Footnotes as in Appendix A.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
24 h p.e.				
Proportion of	Ia	$\chi^2_{me}=0{\cdot}15$, $P=0{\cdot}70$, 1 D.F.	$\chi^2_{mo}=39{\cdot}61$, $P\ll 0{\cdot}001$, 1 D.F.	$\chi^2_{eo}=36{\cdot}47$, $P\ll 0{\cdot}001$, 1 D.F.
flies with	IIa			Fisher exact test, $P=0{\cdot}98$
early L1	IIb	$\chi^2_{me}=0{\cdot}04$, $P=0{\cdot}84$, 1 D.F.	$\chi^2_{mo}=22{\cdot}85$, $P\ll 0{\cdot}001$, 1 D.F.	$\chi^2_{eo}=34{\cdot}06$, $P\ll 0{\cdot}001$, 1 D.F.
No. of early	Ia	$H_{me}=1{\cdot}06$, $P=0{\cdot}30$, 1 D.F.	$H_{mo}=41{\cdot}30$, $P\ll 0{\cdot}001$, 1 D.F.	$H_{eo}=37{\cdot}62$, $P\ll 0{\cdot}001$, 1 D.F.
L1/fly	IIa			$H_{eo}=3{\cdot}74$, $P=0{\cdot}053$, 1 D.F.
	IIb	$H_{me}=0{\cdot}008$, $P=0{\cdot}996$, 1 D.F.	$H_{mo}=26{\cdot}33$, $P\ll 0{\cdot}001$, 1 D.F.	$H_{eo}=35{\cdot}13$, $P\ll 0{\cdot}001$, 1 D.F.

Appendix D. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* microfilarial intake among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
6–10 days p.e.				
Proportion of	Ia	$\chi^2_{me}=0{\cdot}08$, $P=0{\cdot}78$, 1 D.F.	$\chi^2_{mo}=40{\cdot}85$, $P\ll 0{\cdot}001$, 1 D.F.	$\chi^2_{eo}=28{\cdot}27$, $P\ll 0{\cdot}001$, 1 D.F.
flies with L1,	IIa			$\chi^2_{eo}=5{\cdot}32$, $P\ll 0{\cdot}02$, 1 D.F.
L2, L3	IIb	$\chi^2_{me}=0{\cdot}002$, $P=0{\cdot}96$, 1 D.F.	$\chi^2_{mo}=54{\cdot}85$, $P\ll 0{\cdot}001$, 2 D.F.	$\chi^2_{eo}=39{\cdot}97$, $P\ll 0{\cdot}001$, 1 D.F.
No. of	Ia	$H_{me}=0{\cdot}07$, $P=0{\cdot}79$, 1 D.F.	$H_{mo}=40{\cdot}25$, $P\ll 0{\cdot}001$, 1 D.F.	$H_{eo}=29{\cdot}90$, $P\ll 0{\cdot}001$, 1 D.F.
all larval	IIa			$H_{eo}=9{\cdot}17$, $P=0{\cdot}002$, 1 D.F.
stages/fly	IIb	$H_{me}=0{\cdot}001$, $P=0{\cdot}98$, 1 D.F.	$H_{mo}=55{\cdot}87$, $P\ll 0{\cdot}001$, 1 D.F.	$H_{eo}=43{\cdot}69$, $P\ll 0{\cdot}001$, 1 D.F.

Appendix E. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* developing larvae (L1 and L2) among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus. Footnotes as in Appendix A.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
6–10 days				
Proportion of	Ia	$\chi^2_{me}=1\cdot65$, $P=0\cdot20$, 1 D.F.	$\chi^2_{mo}=23\cdot01$, $P\ll0\cdot001$, 1 D.F.	$\chi^2_{eo}=28\cdot27$, $P\ll0\cdot001$, 1 D.F.
flies with L1	IIa			$\chi^2_{eo}=34\cdot85$, $P\ll0\cdot001$, 1 D.F.
and L2	IIb	$\chi^2_{me}=0\cdot04$, $P=0\cdot84$, 1 D.F.	$\chi^2_{mo}=60\cdot82$, $P\ll0\cdot001$, 1 D.F.	$\chi^2_{eo}=51\cdot02$, $P\ll0\cdot001$, 1 D.F.
No. of	Ia	$H_{me}=3\cdot42$, $P=0\cdot06$, 1 D.F.	$H_{mo}=24\cdot26$, $P\ll0\cdot001$, 1 D.F.	$H_{eo}=32\cdot11$, $P\ll0\cdot001$, 1 D.F.
developing	IIa			$H_{eo}=44\cdot64$, $P\ll0\cdot001$, 1 D.F.
larvae/fly	IIb	$H_{me}=0\cdot018$, $P=0\cdot89$, 1 D.F.	$H_{mo}=60\cdot48$, $P\ll0\cdot001$, 1 D.F.	$H_{eo}=53\cdot44$, $P\ll0\cdot001$, 1 D.F.

Appendix F. Values of the chi-square (χ^2) and Kruskal–Wallis (H) tests, associated probabilities (P) and degrees of freedom (D.F.) for comparisons of *Onchocerca volvulus* infective larvae (L3) among *Simulium metallicum s.l.* (north-eastern Venezuela), *Simulium exiguum s.l.* and *Simulium oyapockense s.l.* (Amazonas region) in cross-experimental infections. Subject I is the carrier of northern Venezuelan parasites; Subject II harbours infection from the Amazonian focus. Footnotes as in Appendix A.

Blackfly species (cibarium)…		*S. metallicum* (*m*) (unarmed)	*S. exiguum* (*e*) (unarmed)	*S. oyapockense* (*o*) (armed)
Locality…	Subject	Carrasposo Anzoátegui State	Culebra and Alto Carinagua Puerto Ayacucho, Amazonas State	
6–10 days p.e.				
Proportion of	Ia	$\chi^2_{me}=3\cdot95$, $P\ll0\cdot047$, 1 D.F.	$\chi^2_{meo}=20\cdot52$, $P\ll0\cdot001$, 2 D.F.	
flies with			$\chi^2_{mo}=14\cdot05$, $P<0\cdot001$, 1 D.F.	
L3 larvae/fly	IIa			$\chi^2_{eo}=4\cdot33$, $P=0\cdot04$, 1 D.F.
	IIb		$\chi^2_{meo}=1\cdot87$, $P=0\cdot39$, 1 D.F.	
No. of	Ia	$H_{me}=5\cdot22$, $P=0\cdot02$, 1 D.F.	$H_{meo}=20\cdot22$, $P\ll0\cdot001$, 2 D.F.	
infective			$H_{mo}=15\cdot66$, $P<0\cdot001$, 1 D.F.	
larvae/fly	IIa			$H_{eo}=5\cdot50$, $P\ll0\cdot019$, 1 D.F.
	IIb		$H_{meo}=1\cdot86$, $P=0\cdot39$, 2 D.F.	

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